1 **UV-C and wet heat resistance of *Bacillus thuringiensis* biopesticide endospores compared to**
2 **foodborne *Bacillus cereus* endospores**

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Abstract

Bacillus endospores (spores) are generally resistant to environmental and food processing-related stress including thermal and non-thermal processing in the food industry, such as pasteurization, and UV-C inactivation. *Bacillus thuringiensis* insecticidal crystals and spores as the active substances in commercial biopesticides can also be introduced to vegetable foods and their food processing environment due to pre-harvest treatment of edible crops. The resistance of *B. thuringiensis* biopesticide spores in comparison to the genetically closely related foodborne *B. cereus* against heat and UV-C treatment is investigated in this study. The results show that *B. thuringiensis* biopesticide spores with the commercial granulated product formulation are better protected and as such more resistant to both wet heat (D values at 90°C: 50.1-79.5 min) and UV-C treatment (D values at 0.6 mW/cm^2: 7.5-8.9 min) than the pure spore suspension. The enhanced UV-C resistance properties of *B. thuringiensis*-formulated spores also indicate that the *B. thuringiensis* spores in powder or granule formulation applied in the field might not be effectively inactivated by solar radiation (UV-A and UV-B) in a short period. Furthermore, the spores of one emetic *B. cereus* toxin-producing strain (LFMFP 254; a Belgian outbreak strain) were found more resistant to the wet heat at 90°C (D_{90}-value=71.2 min) than other tested pure spore suspensions, although the spores of *B. cereus* 254 did not show different behavior against UV-C treatment. This result suggests that UV-C treatment can be applied as an effective inactivation method against *B. cereus* 254 spores.

Keywords: *B. thuringiensis* biopesticides, *B. cereus*, endospore, UV-C resistance, heat resistance

55 **1 Introduction**

56 In order to extend the shelf stability and assure the safety of food products, many thermal and non-
57 thermal technologies are used in the food industry (Boateng, 2022; Jayathunge et al., 2019). As a non-
58 thermal technology, ultraviolet light C-region (UV-C) at a wavelength of 254 nm is considered an
59 effective microbial decontamination technology due to its germicidal and sporicidal properties (Gayán
60 et al., 2013; Gómez-López et al., 2007; Guerrero-Beltrán and Barbosa-Cánovas, 2004; Rajkovic et al.,
61 2017). UV-C processing is a rapid disinfection technology with low cost, generates no harmful chemical
62 residues, and causes no significant product heating (Gómez-López et al., 2007; Guerrero-Beltrán and
63 Barbosa-Cánovas, 2004). However, the drawback of UV-C treatment is its low penetration capacity,
64 sensitivity to shadowing effect and possible long treatment times, which limits the antimicrobial
65 efficacy (Gabriel et al., 2016) in foods. Therefore, UV-C has been most often used to disinfect air, water,
66 food contact surfaces and decontaminate liquid food, lower surface contamination of carcasses or
67 foods of plant origin (Bintsis et al., 2000; Gómez-López et al., 2007).

68 Thermal processing describing all forms of heat treatment including sterilization, pasteurization,
69 microwave heating, etc. has been commonly used for a long time. Thermal processing is one of the
70 most important technologies to provide the required level of food safety and prolong food shelf life by
71 eliminating spoilage microorganisms and pathogens in food, as well as inactivating enzymes and some
72 microbial metabolites (Sun, 2012). It is generally accepted that mild heat treatment of cooked chilled
73 food at 70 °C for 2 min and 90°C for 10 min achieves a 6 log (6D) reduction of *Listeria monocytogenes*
74 and nonproteolytic *Clostridium botulinum*, respectively (Bergis et al., 2021; Katherine Scurrah, 2010).
75 Besides *C. botulinum*, another key target psychrotolerant pathogenic spore-former is *Bacillus cereus*
76 *sensu lato* (*s. l.*) or *B. cereus* group, which must be controlled in the preparation and storage of cooked
77 chilled food (Daelman et al., 2013).

78 A survival strategy of *B. cereus s. l.* cells is to produce endospores (spores) which are highly specialized,
79 metabolic dormant, and resistant to extreme environmental stresses. If the environmental conditions
80 become favorable, spores can germinate in the presence of germinating agents and grow out to reach
81 a considerable number in different environments including processed foods (Abee et al., 2011). The
82 spore consists of a core surrounded by an inner membrane, a core wall (also known as a germ cell wall),
83 a cortex, an outer membrane, a coat layer, and some spores even possess an exosporium surrounding
84 the coat layer (Setlow et al., 2017). The structure and biochemical composition reveal that not only do
85 the multi-layers protects spores to survive under the harsh conditions, but also the germinant
86 receptors enable spores to detect their favorable specific germinants and then germinate (Abee et al.,
87 2011; Moir and Cooper, 2015). The common germinants include nutrient germinants such as sugars,

88 amino acids (e.g. L-alanine), purine nucleosides, inorganic salts, and non-germinant receptor-
89 dependent germinants such as Ca^{2+}-dipicolinic acid (CaDPA) or dodecylamine. However, spore
90 germination can also be triggered by receptor-independent processes such as physical effectors
91 including heat shock and high pressure (Abee et al., 2011; Moir and Cooper, 2015).

92 As one of the widely used biological control agents (BCAs) in the world, *B. thuringiensis*-based
93 biopesticides were registered with more than 400 formulations containing insecticidal proteins and
94 viable spores in the market (Jalali et al., 2020). These formulations are produced to improve the
95 efficiency of *B. thuringiensis*-based products applied in the field, i.e. to increase the persistence and
96 stability of the excreted (crude) Cry-toxin responsible for insecticidal activity after application to the
97 plants. Three tested *B. thuringiensis* commercial products (XenTari®, DiPel® and Delfin®) in our study
98 are wettable granules that 'partially encapsulated' dried Cry-toxin crystals and *B. thuringiensis* spores
99 with different materials such as diatomaceous earth, talc and zeolites from Valent BioSciences or
100 Biobest. The filling powders and the active substances are stuck/pressed/limed together into granules
101 to prevent dust formation from the original powder (Townsend, 2018).Spores and insecticidal crystals
102 of *B. thuringiensis* as active substances in the commercial biopesticide can be introduced to edible
103 plants for controlling the insects (i.e. Lepidoptera) during pre-harvest operations and be present in
104 final foods derived thereof. It has been reported that some outbreaks seem to associate with *B.*
105 *thuringiensis* biopesticide strains in salads or dishes containing vegetables (Bonis et al., 2021; EFSA,
106 2016), whereas the magnitude of the risk of using *B. thuringiensis* biopesticides on edible plant is
107 debatable and raises many concerns in food safety. The spores of biopesticidal *B. thuringiensis* can also
108 enter and persist in the food processing environment or form biofilm on the food contact surface (Zhao
109 et al., 2022), and these spores might be a challenge for the industrial cleaning regime.

110 *B. cereus* group comprises 24 validated species (Parte et al., 2020) that are genetically closely related
111 to each other. *B. cereus* group is an etiological agent of two types of foodborne diseases: food
112 intoxication caused by emetic toxin-producing members and toxico-infection caused by diarrheal
113 toxin-producing members including *B. thuringiensis* (Biggel et al., 2022; De Bock et al., 2021; Jovanovic
114 et al., 2021). However, in routine food diagnostics, *B. thuringiensis* cannot be differentiated from other
115 *B. cereus* group members using standard selective agar (i.e. Mannitol Egg Yolk Polymyxin (MYP) agar),
116 and will be identified 'presumptive *B. cereus*'(De Bock et al., 2021; ISO 7932, 2020) . To understand the
117 behavior of biopesticidal *B. thuringiensis* spores against the often-used thermal and non-thermal
118 inactivation technologies in the food industry, wet heat (at 90 °C) and UV-C treatments (at 254 nm
119 with the fluence rate of 0.6 mW/cm^2) were selected in this study. In addition, some other foodborne
120 *B. cereus s. l.* strains (particularly the diarrheal toxin producers) were selected as the comparisons to
121 glimpse the role of *B. thuringiensis* biopesticide strains in the *B. cereus* group.

122 **2 Materials and methods**

123 **2.1 Bacterial strains**

124 The *B. thuringiensis* biopesticide strains were isolated from the XenTari® WG, DiPel® WG and Delfin®
125 WG granules by plating the dissolved products on Mannitol Egg Yolk Polymyxin (MYP) agar (Oxoid)
126 plates and incubating for 24 h at 30°C. All *B. cereus* group strains were maintained at -75°C in 15% or
127 20% glycerol for long-term storage. The strains were revived in 9 mL brain heart infusion (BHI; Oxoid)
128 broth overnight at 30°C. Loops of the overnight cultures were streaked on Mannitol Egg Yolk Polymyxin
129 (MYP) agar (Oxoid) plates and incubated at 30°C for 18-24 h. After the incubation, the MYP agar plates
130 were stored at 4°C as the work stocks for a maximum of 4 weeks. The strains used in this study are
131 summarized in Table 1.

132 **2.2 Spore suspension preparation**

133 The spore suspension harvested from strengthened Nutrient Agar (sNA; 28 g/L Nutrient Agar (Oxoid)
134 + 0.04 g/L $MgCl_2$ (Sigma-Aldrich) + 0.10 g/L $CaCl_2$ (Sigma-Aldrich)) plates was performed according to
135 Samapundo et al. (2011) with little modification. However, *B. cereus* LFMFP 836 proved difficult to
136 sporulate on sNA using the previously mentioned protocol, thus the spores of this strain were prepared
137 based on the methods described by Begyn et al. (2020) using maltose sporulation medium (MSM). The
138 MSM consists of the following components: 5g/L Difco™ nutrient broth (Becton Dickinson, USA), 10
139 mM maltose, 1 µM $FeSO_4$, 1 mM $MgCl_2{\cdot}6H_2O$, 1 mM $Ca(NO_3)_2{\cdot}4H_2O$, 12.5 µM $CuCl_2{\cdot}2H_2O$, 2.5 µM
140 $CoCl_2{\cdot}6H_2O$, 2.5 µM $Na_2MoO_4{\cdot}2H_2O$, 66 µM $MnSO_4{\cdot}H_2O$, 12.5 µM $ZnCl_2$, 5 mM $(NH_4)_2SO_4$ (Garcia et al.,
141 2010). Vegetative cells were eliminated using ethanol-water (1/1) and incubated at 2°C for 1-2 h. The
142 final spore suspension stock (ca. > 99% are spores and > 90% are phase bright) was suspended in
143 100mM sodium phosphate buffer (pH 7.4) with 0.01% Tween 80 and was stored at 2°C, and then used
144 within 4 weeks. The concentration (10^8-10^9 CFU/mL) was verified by spread-plating on TSA plates.

145 Except for lab-harvested spores, spore suspensions prepared by the *B. thuringiensis* commercial
146 products were also investigated. One gram of granules of the commercial products (XenTari®, DiPel®,
147 and Delfin®) were dissolved in 30 mL sterile distilled water (~10^9cfu/mL of spores) under the agitation
148 condition for 30 min, and the concentration was also checked by spread-plating on TSA plates.

149 **2.3 UV-C treatment**

150 UV-C treatment of spores was performed according to Begyn et al. (2020) with some modifications.
151 This experiment was performed in a closed chamber with a UVpro K17-2 lamp (BioClimatic, The
152 Netherlands) on the top, and the distance between the lamp and sample (bottom) was 19.4 cm (Fig.
153 S1). A radiometer (ILT 1700) connected to XRD1407254 detector (International Light Technologies,

154 Peabody, USA) was used to measure the UV-C doses. Before each sample treatment, the UV-C lamp
155 was allowed to warm up until stabilization by turning it on for 10 min (Fig. S2), and the treatment
156 started at 11th min. The UV-C exposure doses to samples ranged from 36 to 252 mJ/cm^2 corresponding
157 with a treatment duration of 1 to 7 min.

158 The spore suspension prepared in 2.2 was diluted in 100mM sodium phosphate buffer (pH 7.4) with
159 0.01% Tween 80 until reaching 10^8 CFU/mL spore concentration. Subsequently, 8 mL of the spore
160 suspension obtained above was transferred into a petri dish (Ø55mm) with a sterilized magnetic stir
161 bar. The depth of the spore suspension in the petri dish is ca. 3.4 mm. The petri dish was located in the
162 center of the bottom with the lid open, and spores were continuously stirred during the UV-C exposure
163 on a magnetic stirrer (RCT basic, IKA, China) setting the speed at ca. 750 rpm. After the treatment, the
164 lamp was turned off and cooled down for 15 min for the next treatment, and the surviving spores in
165 the petri dish were serially diluted in PPS with 0.01% Tween 80 and plated on TSA after 24 h incubation
166 at 30°C for enumeration. In addition, the initial spore concentration was counted both before and after
167 a heat treatment at 80°C for 10 min, to check if vegetative cells or geminated spores existed or not.
168 The prior heat treatment was only applied to one independent sample, not to the whole spore
169 suspension in stock.

170 **2.4 Heat treatment**

171 A 50 µL aliquot of the spore suspension (both pure spore suspensions and commercial granule
172 suspensions) with an initial concentration of 10^8 CFU/mL was transferred into a 0.2 mL sterile ultra-
173 thin walled PCR tube (BIOplastics, The Netherlands). Heat treatment of spores was performed in a PCR
174 thermocycler (ThermoFisher, USA) at 90°C with the lid temperature at 105°C with the following heat
175 block protocol: started at 25°C for 15 seconds to balance the temperature differences, then increase
176 the temperature to 90°C for 10, 30, 60, 90, or 120 minutes (each timepoint a separate run), and finally,
177 cooled at 4°C immediately to prevent germination. After the heat treatment, the surviving spores were
178 serially diluted in PPS with 0.01% Tween 80 and plated on TSA for enumeration. At t_0, one independent
179 sample of the spore suspension was counted both before and after a heat treatment at 80°C for 10
180 min in the water bath to check if vegetative cells and germinated spores exist or not.

181 The same experiment set up for vegetative cells of tested strains was also performed at 90°C. The
182 vegetative cell suspensions were obtained from the second overnight culture, centrifuged and
183 resuspended in PPS + 0.01% Tween 80, and the initial concentrations of heat treatment also all started
184 at 10^8 CFU/mL. The absence of spores were confirmed under phase-contrast microscopy (ZEISS
185 Axioscope 5, Germany). As more heat-sensitive properties of vegetative cells, the treatment periods
186 changed into 1, 2, 5, 10 and 20 min.

187 **2.5 Data analysis**

188 Six pure spore suspensions harvested using sNA and *B. cereus* 836 pure spore suspension prepared
189 using MSM, as well as 3 commercial *B. thuringiensis* granule suspensions (XenTari®, DiPel®, and Delfin®)
190 were tested for both UV-C and wet heat treatment. Vegetative cells of seven strains as listed in Table
191 1 were tested only for wet heat treatment. All experiments were performed three times on three
192 different days for three biological replicates, and the means of triplicate data points were analyzed
193 using GInaFiT inactivation model-fitting tool (Geeraerd et al., 2005).

194 For UV-C and heat inactivation, different models of tested strains were fitted including log-linear, log-
195 linear + tail and shoulder + log-linear, the equations are expressed in Table 2.

196 In the equations, N is the counts (CFU/mL) of survivors after exposure time t; N_0 and N_{res} represent the
197 initial and residual population (CFU/mL), respectively; k_{max} (1/min) is the maximum specific inactivation
198 rate; S_l donates the shoulder period. D is determined as the time required to cause a 90% population
199 reduction (1 log10 reduction) of the microbial count.

200 D-values (min) for both heat and UV-C inactivation for each strain were calculated based on the
201 transformed equation below:

202 $$D = \frac{\ln(10)}{k_{max}} + S_l$$

203 When log-linear or log-linear + tail model was identified, S_l equaled to 0.

204 In addition, statistical analysis was performed in SPSS® version 28 (IBM, USA). The log reductions at
205 each exposure time point among strains were analyzed by one-way ANOVA followed by Tukey HSD or
206 Games-Howell post hoc test for equal variances or unequal variances, respectively. Statistical
207 differences of D values among strains were also analyzed by the same way above. A statistically
208 significant difference was indicated by a p-value of less than 0.05.

209 **3 Results**

210 **3.1 UV-C inactivation of spores**

211 With the increase of UV-C (0.6 mW/cm^2) exposure time from 1 min to 7 min and the corresponding
212 delivered UV-C dose of 36 to 252 mJ/cm^2 (Fig. 1), the survival spores of tested strains followed different
213 inactivation models (Fig. S3). The inactivation model of three *B. thuringiensis* commercial granule
214 suspensions (XenTari®, DiPel® and Delfin®) followed the shoulder + log-linear model, the UV-C
215 inactivation curves of xentari, dipel, delfin, *B. thuringiensis* 464, *B. cereus* 836 and *B. cereus* 257 spores
216 fitted log-linear + tail model, and only *B. cereus* 254 spores data were found to fit log-linear model. The

217 log reductions at each time point and the significant differences among strains are shown in Fig. 2A.
218 From 1 to 7 min (corresponding UV-C exposure from 36 to 252 mJ/cm^2), significantly fewer ($p < 0.05$)
219 log reductions of three *B. thuringiensis* commercial granule suspensions than the other pure spore
220 suspensions were observed. However, only at the UV-C exposure of 1 min (36 mJ/cm^2), significant
221 differences ($p < 0.05$) were found among 7 pure spore suspensions. From UV-C exposure time of 2 to
222 7 min (corresponding UV-C exposure from 72 to 252 mJ/cm^2), no significant differences ($p > 0.05$) were
223 found among 7 pure spore suspensions. After 7 min exposure to UV-C (252 mJ/cm^2), less than 1 log
224 reduction of XenTari®, DiPel® and Delfin® were found, but ca. 4-5 log reductions of the other 7 lab-
225 harvested pure spore suspensions were noted.

226 D values for UV-C treatment taking account into the shoulder length (S_l) are shown in Table 3. D_{UV}-
227 values of spores in commercial *B. thuringiensis* granule suspensions ranged from a mean of 7.5 to 8.9
228 min, but D_{UV}-values of pure spores only ranged from a mean of 0.4 to 1.1 min. Significantly higher ($p <$
229 0.001) D_{UV}-values of spores in XenTari®, DiPel® and Delfin® suspensions than all tested pure spore
230 suspensions were detected. However, no significant difference ($p > 0.05$) of D_{UV}-values was found in
231 any pair of tested pure spores including the pure spore of xentari, dipel and delfin.

232 **3.2 Heat inactivation of spores and vegetative cells**

233 Spores in three tested *B. thuringiensis* commercial granule suspensions were found to have
234 significantly higher ($p < 0.001$) D_{90}-values (mean: 50.1-79.5 min) than the pure spores (mean: 12.9-18.4
235 min) except for *B. cereus* 254. The D_{90}-value of *B. cereus* 254 spores (mean: 71.2 min) was significantly
236 higher ($p < 0.001$) than the other six tested pure spores (Table 3).

237 The log reductions treated with wet heat at 90°C at each time point and the significant differences
238 among strains are shown in Fig. 2B. From 30 to 120 min, significantly fewer ($p < 0.001$) inactivated
239 spores were found in XenTari®, DiPel®, Delfin® commercial granule suspensions and *B. cereus* 254
240 spore suspension than in the rest of six tested spore suspensions. After heat treatment at 90°C for 120
241 min, ca. 6D (6 log reductions) was achieved for xentari, dipel, delfin, *B. thuringiensis* 464, *B. cereus* 836
242 and *B. cereus* 257 spores, but only ca. 2D was found for XenTari®, DiPel®, Delfin®, and ca. 3D was noted
243 for *B. cereus* 254.

244 In order to check if the vegetative cells of *B. cereus* 254 are also resistant to heat treatment or not, the
245 wet heat treatment (at 90°C for 1, 2, 5, 10 and 20 min) for vegetative cells was performed. All tested
246 strains fitted the log-linear + tail inactivation model in GInaFiT (graphs not shown), D_{90}-values of
247 vegetative cells were calculated presented in Table 3. The D_{90}-values of vegetative cells ranged from a
248 mean of 0.3 to 0.5, and no any significant difference ($p > 0.05$) was detected among strains (Table 3).
249 Thus, the log reductions after each exposure period are shown in Fig. 3. At 1 and 5 min, the vegetative

cells of *B. cereus* 254 showed significantly lower ($p < 0.05$) log reductions than the vegetative cells of the other tested strains, except for delfin at 1 min. After exposure for 2 and 10 min, some significant differences between *B. cereus* 254 and other strains were also found, but not with all. However, it is worth noting that most significant differences are less than 1 log reduction. An 8-log decrease in 20 min at 90°C for the vegetative cells was noted, and no significant differences ($p > 0.05$) were observed for the vegetative cells in any comparisons of tested strains. It is also worth noting that after the heat treatment at 90°C for 10 min, the mean log reductions of all vegetative cells reached more than 6D.

4 Discussion

The formulation of *B. thuringiensis* commercial granule protects the *B. thuringiensis* spores inside against UV-C radiation and heat treatment, but the corresponding pure spore suspensions harvested in the lab are not found with more resistant properties against UV-C and heat treatment (90°C) than the other tested *B. thuringiensis* or *B. cereus* spores. Our results of both D-values and the log reductions reached at each time point (Table 3 & Fig. 2) all indicate more resistance of *B. thuringiensis* spores with commercial formulation against either UV-C or heat treatment (90°C) than the pure spore suspensions harvested in the lab. Many researchers have studied the enhanced UV protection of different *B. thuringiensis* formulations (Behle et al., 2011; Jalali et al., 2020; Jallouli et al., 2014; Tamez-Guerra et al., 2000), but mainly focused on the UV radiations from the sunlight (UV-A and UV-B) for the efficient application in the field (pre-harvest) instead of the artificial UV-C radiation (254 nm) as often used as non-thermal inactivation technology in food processing industry. To our knowledge, this is also the first time to report the enhanced heat resistance of *B. thuringiensis*-formulated spores. In addition, our study was the first to report that unformulated *B. thuringiensis* biopesticide spores did not show more resistant properties against UV-C and heat treatment (90°C) than the other tested *B. thuringiensis* or *B. cereus* spores.

The spore form of one emetic toxin-producing strain (*B. cereus* LFMFP 254) from a Belgian outbreak was found to be significantly more resistant to the wet heat at 90°C than other tested unformulated spore suspensions, but the spore of *B. cereus* 254 did not show different behavior against UV-C treatment than other tested unformulated spores. Previously published D_{90}-values of *B. cereus* spores in distilled water or PPS with 0.01% Tween 80 were variable ranging from 4.04 min to 39 min (Fernández et al., 2001; Kim et al., 2021; Valero et al., 2006). Our results of D_{90}-values of tested *B. thuringiensis* and *B. cereus* pure spores (12.9-18.4 min) are in agreement with the published results mentioned above, except for the *B. cereus* 254 spore. Our results show that the D_{90}-value of *B. cereus* 254 spore is 71.2 min which is significantly higher than the D_{90}-values of other tested *B. thuringiensis* and *B. cereus* spores (Table 3). This *B. cereus* 254 strain was isolated from one sample of the pasta

283 salads in a fatal family outbreak that happened in 2003, and the highest *B. cereus* count was detected
284 in the pasta salad ranging from 10^7 to 10^8 CFU/g (Dierick et al., 2005). One hypothesis of the enhanced
285 heat resistance of *B. cereus* 254 spore is considered that the spores are survivors after the heating
286 during the preparation of the pasta for the salads at home, and the surviving spores evolved to obtain
287 enhanced heat resistance. A similar study has recently reported the reproducible evolution of *B.*
288 *weihenstephanensis* endospore for increased heat resistance (Kim et al., 2021). In addition, it has been
289 reported by other researchers that emetic *B. cereus* spores had higher resistance to heat than diarrheal
290 *B. cereus* spores (Carlin et al., 2006; Kwon et al., 2019). However, the enhanced resistance property of
291 *B. cereus* 254 spore was only found against heat treatment at 90°C rather than UV-C treatment (36 to
292 252 mJ/cm^2). The different behaviors of *B. cereus* 254 spores can be explained by the different
293 inactivation mechanisms of spores by UV-C and wet heat treatment. The mechanism of microbial
294 inactivation by UV-C is mainly regarded as damage to DNA synthesis, and then a minor reason is
295 considered as damage to membranes, proteins and other macromolecules (Gómez-López et al., 2007).
296 For the spore resistance to wet heat, the major intrinsic factor is the core water content: a lower core
297 water content leads to reduced molecular mobility of core proteins and results in elevated higher
298 protein resistance to the wet heat (Setlow, 2014). In addition, many sporulation parameters i.e. solid
299 or liquid sporulation media, divalent metal ions, and sporulation temperature have an important effect
300 on heat resistance (Abhyankar et al., 2016; MAZAS et al., 1995). However, in our study, *B. cereus* 836
301 spore which is the only one harvested by the MSM liquid medium did not show significant differences
302 between the other spores harvested from sNA agar medium (Fig.2). **Vegetative cells of *B. cereus* 254**
303 **did not show such increased wet heat resistance as its spores.** Although some significant differences
304 were observed between *B. cereus* 254 and vegetative cells of other tested strains in some short
305 exposure time, its D_{90}-value is quite close to the others as described above. Furthermore, after the
306 wet heat treatment at 90°C for 10 min, all tested vegetative cells reached more than 6 log reductions.
307 However, this conclusion could become different if the wet heat treatment temperature changed, i.e.
308 70 or 80 °C or even lower temperature applied.

309 **5. Conclusions**

310 Our study reveals that the standard mild thermal pasteurization process (90°C, 10 min) is effective for
311 inactivation of *B. cereus s. l.* vegetative cells, but leads only to a maximum 1 log reduction of the spores.
312 Application of granulated formulations of *B. thuringiensis* biopesticides in the pre-harvest operations
313 results in *B. thuringiensis* presence in the post-harvest stages, and in final foods in a form that is even
314 more wet heat and UV-C resistant than the native spores themselves. The higher heat and UV-C
315 resistance of commercially produced biopesticidal *B. thuringiensis* spores, make them difficult to
316 eradicate from food processing environments by hygiene measures. The enhanced UV-C resistance

317 properties of *B. thuringiensis*-formulated spores also indicate that the *B. thuringiensis* biopesticides in
318 powder or granule formulation applied in the field might not be effectively inactivated by the solar
319 radiation (UV-A and UV-B) in a short period. This highlights that the instability and easy degradation
320 properties of '*B. thuringiensis* biopesticides' upon UV or solar radiation might be true for the Cry-toxin,
321 but it is not the case for the *B. thuringiensis* spores in the formulation according to the present study.

322 **Declaration of Competing Interest**

323 The authors declare that they have no known competing financial interests or personal relationships
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471 **Table 1 *B. cereus s. l.* strains used in this study.**

Species	LFMFP strain number	Collection strain number	Origin
B. thuringiensis	xentari	ABTS-1857	XenTari® WG#
	dipel	ABTS-351	DiPel® WG#
	delfin	SA-11	Delfin® WG*
	464	ATCC 10792$	Mediterranean flour moth
***B. cereus* (Diarrhoeal toxin producer)**	836	ATCC 14579$	A farmhouse in the United States in 1916
	257	WIV 5958c	Boiled pasta, Kinrooi outbreak
***B. cereus* (Emetic toxin producer)**	254	LMG 22733 WIV 5964a	Pasta salad, Kinrooi outbreak

472 $ Type strains

473 # Kindly provided by Valent BIoSciences LCC (Libertyville, IL, United States)

474 *Kindly provided by Biobest (Westerlo, Belgium)

475

476 **Table 2 Inactivation models used in this study using GInaFiT freeware tool.**

Model	Equation	Reference
Log-linear	$log_{10}(N) = log_{10}(N_0) - \frac{k_{max}t}{\ln(10)} = log_{10}(N_0) - \frac{t}{D}$	(Bigelow and Esty, 1920)
Log-linear + tail	$log_{10}(N) = log_{10}\left((N_0 - N_{res}) \cdot e^{-k_{max}t} + N_{res}\right)$	(Geeraerd et al., 2000)
Shoulder+ log-linear	$log_{10}(N) = log_{10}(N_0) - \frac{k_{max}t}{\ln(10)} + log_{10}\left(\frac{e^{k_{max}S_l}}{1 + (e^{k_{max}S_l} - 1)} \cdot e^{-k_{max}t}\right)$	(Geeraerd et al., 2000)

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480 **Table 3 D values for *B. thuringiensis* and *B. cereus* spores or vegetative cells from UV-C treatment at**
481 **0.6 mW/cm^2 and wet heat treatment at 90°C.**

Strain	**Spore**				**Vegetative cell**	
	S_l (mean ± SD; min)	**D_{UV}-value (mean ± SD; min)**	**S_l (mean ± SD; min)**	**D_{90}-value (mean ± SD; min)**	**S_l (mean ± SD; min)**	**D_{90}-value (mean ± SD; min)**
XenTari®	5.2±0.2	8.9±3.8^a	27.1±15.4	70.2±12.6^{ab}	-	-
DiPel®	4.6±0.3	7.5±0.9^a	-	50.1±3.6^a	-	-
Delfin®	4.6±2.0	7.9±1.2^a	-	79.5±9.2^b	-	-
xentari	-	0.7±0.2^b	-	15.9±1.0^c	-	0.3±0.1^a
dipel	-	0.6±0.3^b	-	13.0±1.8^c	-	0.4±0.0^a
delfin	-	1.1±0.7^b	-	12.9±0.8^c	-	0.5±0.0^a
Bt 464	-	1.1±0.5^b	-	14.3±1.3^c	-	0.4±0.1^a
Bc 836	-	0.8±0.1^b	-	18.4±1.5^c	-	0.4±0.0^a
Bc 257	-	0.4±0.1^b	-	14.9±4.6^c	-	0.4±0.0^a
Bc 254	-	0.8±0.4^b	44.7±13.5	71.2±9.7^{ab}	-	0.5±0.0^a

482 S_l, shoulder length; SD, standard deviation; Bt, *B. thuringiensis*; Bc, *B. cereus*; -, not applicable.

483 XenTari®, DiPel® and Delfin® were prepared as granule or powder suspensions directly from *B.*
484 *thuringiensis* commercial products, the rests were prepared as pure spore suspensions.

485 Superscripts of D values in each column sharing different letters are significantly different ($p < 0.05$)
486 from each other following the comparisons among strains.

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496 **Figure captions**

497 **Fig. 1. Estimated UV-C doses following time during the UV-C treatment at 0.6 mW/cm^2 against spores.**

498 **Fig. 2. Log reductions of *B. cereus s. l.* spores exposed to UV-C (A) treatment at 0.6 mW/cm^2 and wet**
499 **heat (B) treatment at 90°C following time.** XenTari, DiPel, Delfin represent the commercial granule
500 suspensions, and xentari, dipel and delfin represent the corresponding pure spore suspensions. Bar
501 represents average value ± SD. Bars sharing common small letters are not significantly different ($p \geq$
502 0.05) from each other following the comparisons among strains at the same exposure time. ***
503 indicates all counts from the triplicates were below the limit of detection (2 log CFU/mL), * indicates
504 one out of three counts was below the limit of detection (2 log CFU/mL). Thus log N of the counts
505 below the limit of detection were all regarded as 2 log CFU/mL during the calculation. 'Bt' represents
506 *B. thuringiensis*, 'Bc' represents *B. cereus.*

507 **Fig. 3. Log reductions of *B. cereus s. l.* vegetative cells exposed to wet heat treatment at 90°C**
508 **following time.** Bar represents average value ± SD. Bars sharing common small letters are not
509 significantly different ($p \geq 0.05$) from each other following the comparisons among strains at the same
510 exposure time. *** indicates all counts from the triplicates were below the limit of detection (1 log
511 CFU/mL), ** and * indicate two and one out of three counts were below the limit of detection (1 log
512 CFU/mL), respectively. Thus log N of the counts below the limit of detection were all regarded as 1 log
513 CFU/mL during the calculation. 'Bt' represents *B. thuringiensis*, 'Bc' represents *B. cereus*.

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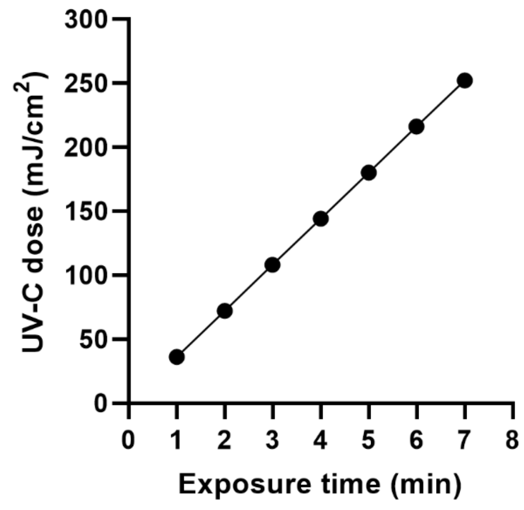

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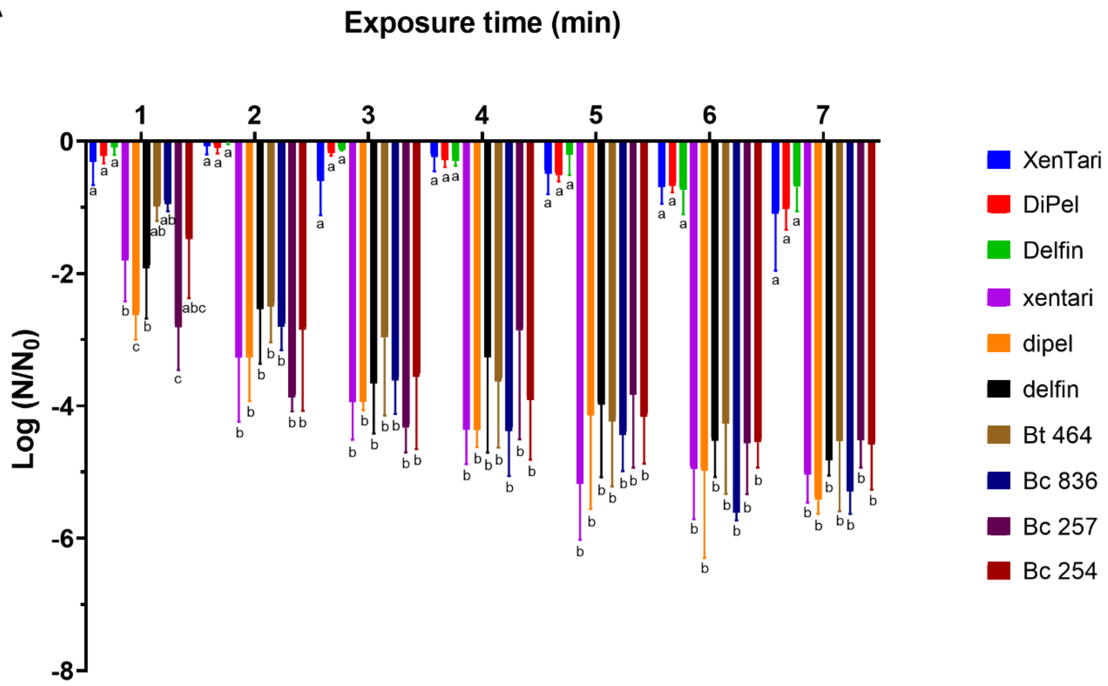
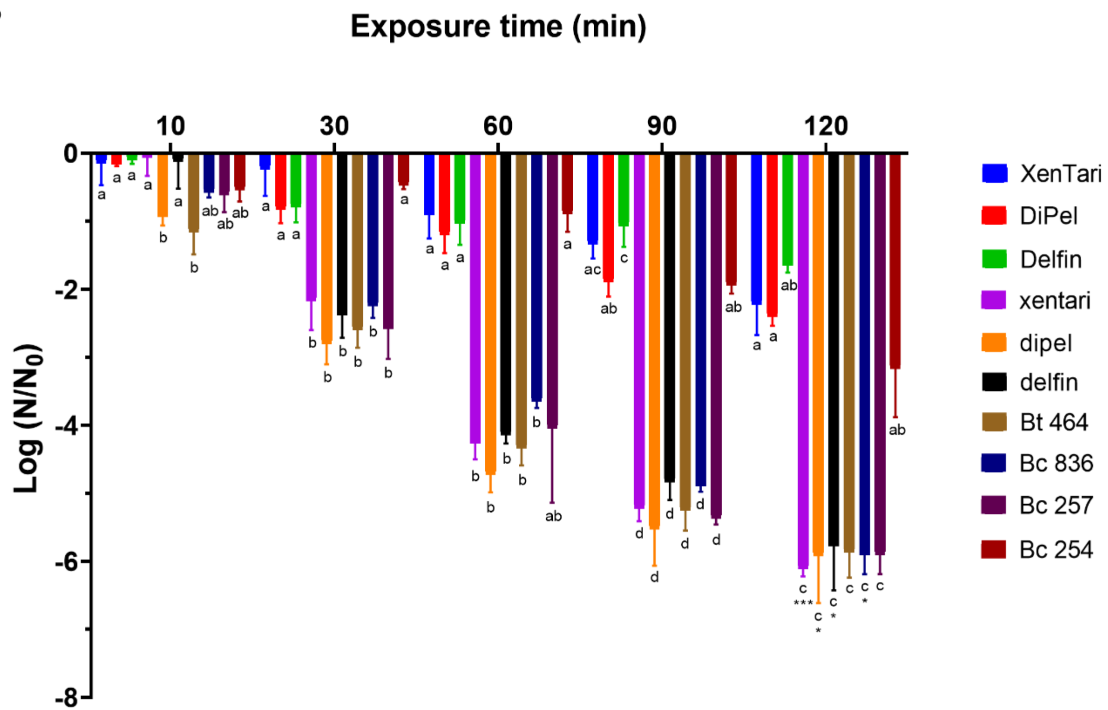
525 Fig. 1





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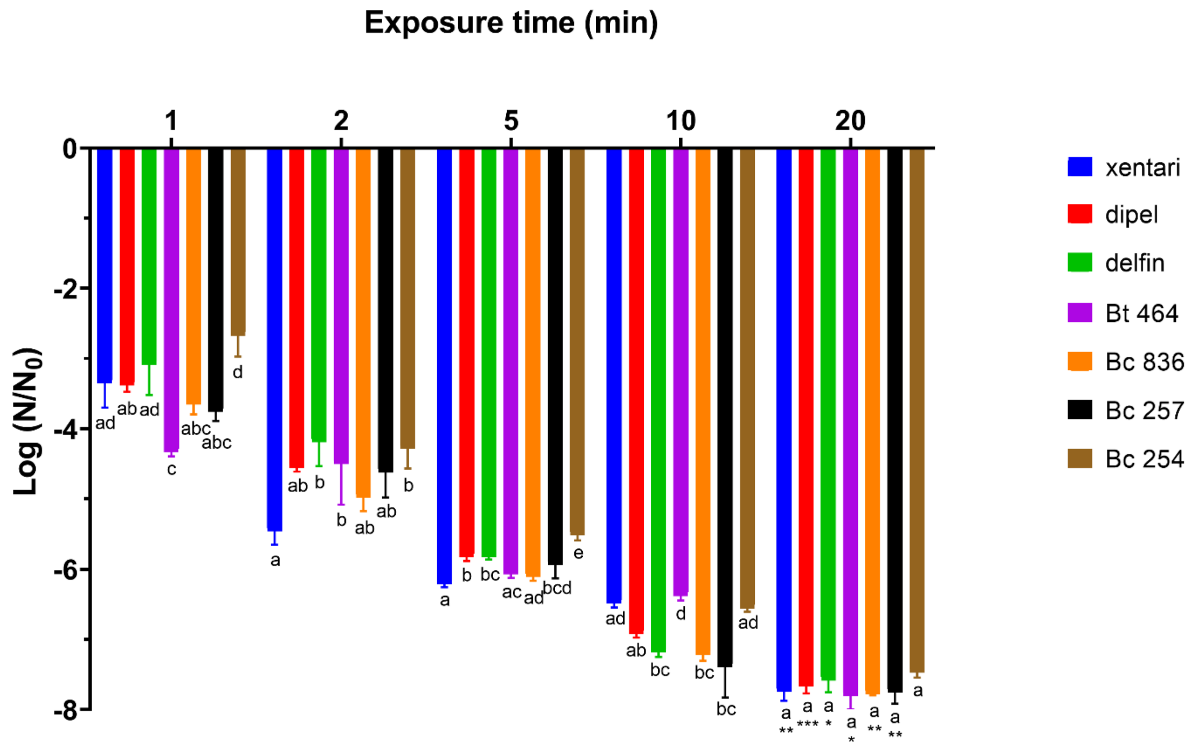
527 **Fig. 2**



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529 **Fig. 3**

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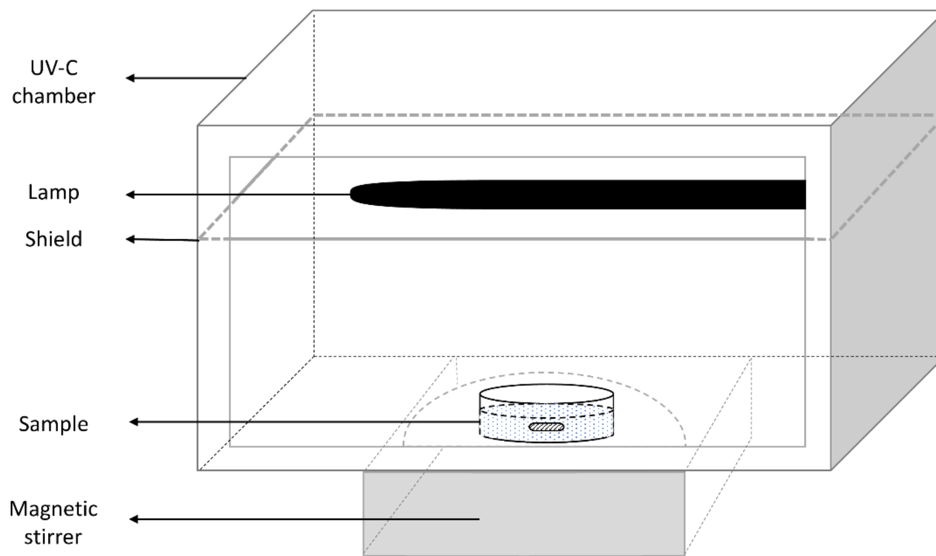
543 **Supplementary materials**



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545 **Fig. S1 Diagram of UV-C chamber and the treatment setting.** The spore suspension sample was in a
546 petri dish (Ø55mm, lid open) with a sterilized magnetic stir bar. The distance between the lamp and
547 sample was 19.4 cm. A shield was put in for the 10-min warm-up period, and pulled out from the 11th
548 min for the UV-C exposure. The speed of the magnetic stirrer was always set at five. Since the lamp
549 was on, the chamber was closed totally. The lamp was cooled with the door of the chamber (not shown
550 in the figure) open for at least 15 min between each treatment.

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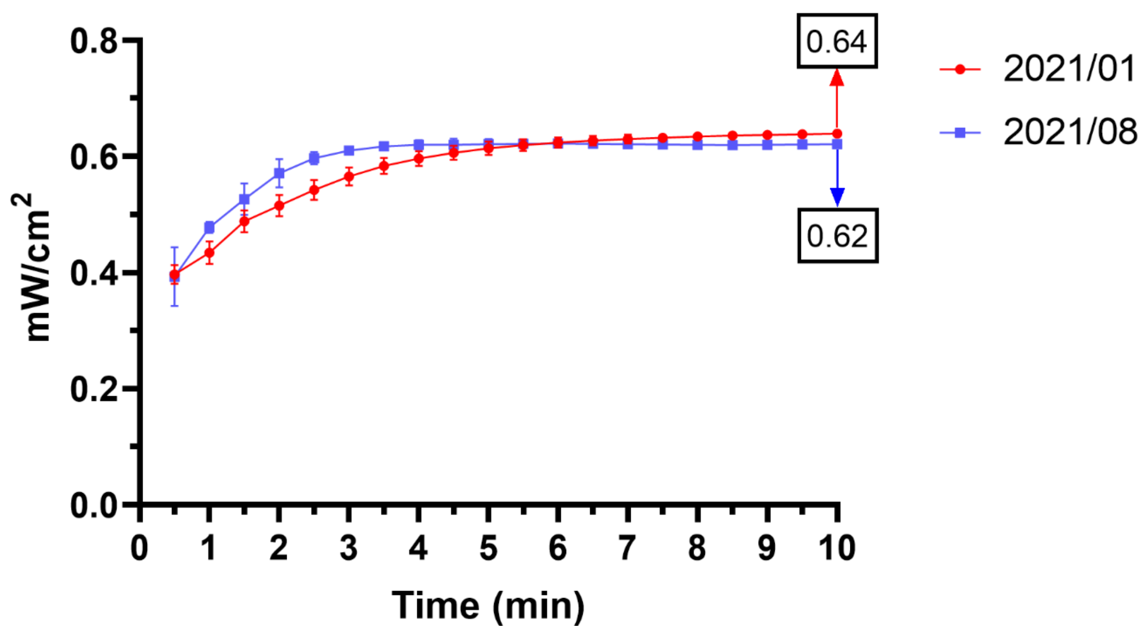

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554 **Fig. S2 Fluence rate of the UV-C lamp over time before and after all experiments in this study.** Each
555 dot represents the mean ± standard deviation (SD) of three measurements performed on the same
556 day. The red and blue lines show the fluence rates of the UV-C lamp in January 2021 and August 2021
557 corresponding to the start and the end of the UV-C experiments, respectively.

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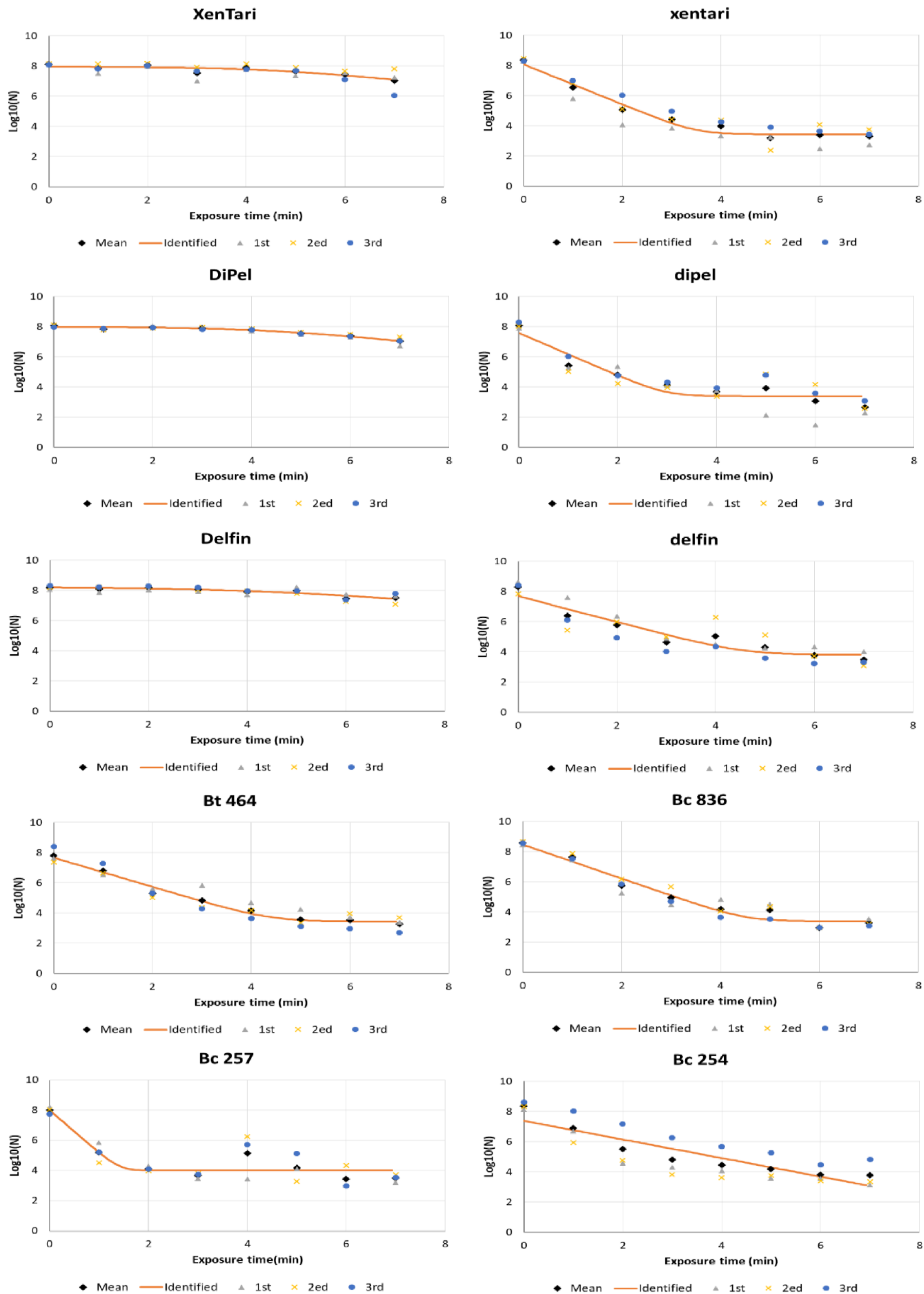

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560 **Fig. S3. Survival curve of spores after the UV-C treatment (0.6 mW/cm^2) following exposure time.**

561 The identified curves were given by GInaFiT fitting different models according to the mean. Three

562 measurements (indicated as symbols labeled with 1st, 2ed and 3rd) were also shown in the plots. 'Bt'

563 represents *B. thuringiensis*, 'Bc' represents *B. cereus*. XenTari, DiPel, Delfin represent the commercial

564 granule suspensions, and xentari, dipel and delfin represent the corresponding pore spore suspensions.

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